



Negative frequency-dependent pollination for rewarding species coincide in natural condition data and agent-based foraging model

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possible titles

should contain:

- Frequency dependence (negative frequency dependence), frequency dependent pollination, frequency dependent selection
- model /agent-based model/ simulation/ foraging model /model data
- natural field condition/ field data/ natural condition /

and maybe something like:

- drivers, reasons, explanation, consistent, coincide, causes, depend, understand
- co-flowering plants, shared pollination, coexistence
- non-linear, sigmoid, cubic

ideas...

- Flower Frequency dependence on pollination...
- Frequency dependent pollination: nonlinear relationship consistent in field and model data
- Understand frequency dependence: Results consistent in natural field conditions and agent-based modeling
- Reasons for positive and negative frequency dependent selection
- Drivers of frequency dependent pollination in natural field condition and agent-based modeling
- Frequency Dependence in natural field conditions and agent-based modeling
- Spatial distribution and cover as limited drivers of flower frequency dependence
- possible causes for frequency dependent pollination of co-flowering plants
- Negative frequency dependence coincide in natural conditions and simulation experiment

Keywords:

- frequency / frequency-dependent
- reproductive success /pollen
- plant-density /density
- flower constancy
- pollinator behavior / foraging behavior
- plant-pollinator interactions
- shared pollination
- coexistence
- spatially-explicit modeling /spatial model / models / individual-based model /agent-based model
- nectar production-rates
- patchy environment

1 Introduction

Frequency-dependence (FD) of survival or reproduction is defined as relative fitness of a species as a function of its frequency in the community (Ayala and Campbell, 1974; Wright and Dobzhansky, 1946). According to ecological theory, frequency dependency could have far-ranging consequences for species coexistence and the maintenance of diversity. Negative frequency dependence occurs if the fitness of a phenotype increases if it is rare and is thought to increase diversity. In the case of plant-pollinator-interactions, negative FD describes the preference of pollinators for the rare flower types which can result in a stable polymorphic equilibrium and an increase in floral diversity. Positive frequency dependence on the other hand defines an increased fitness for the common phenotype. Hence, a higher pollination success for common flower types which tends to reduce diversity in modeling studies (May 1974, but see Bever 1999, Molofsky and Bever 2002). For animal-pollinated plant species, optimal foraging theory predicts that under most circumstances pollinators should favor common flower types over rarer ones (Kunin and Iwasa, 1996).

While the positive effect of density dependence for pollination success is well studied (e.g. Essenberg 2012; Bernhardt et al. 2008; Kunin 1993; Morris et al. 2010, frequency dependence has rarely been tested.

Literature

Previous studies of frequency-dependent pollination cover laboratory, field and modeling experiments.

In the review by Smithson (2001), 11 of 13 lab experiments using artificial flowers on a "bee-board" showed significant results for frequency dependence. 10 of those were done with rewarding flowers and resulted in positive frequency dependence(Smithson and Macnair, 1996, 1997)). The only experiment resulting in negative frequency dependence was done with non-rewarding flowers (Smithson and MacNair, 1997).

The few field experiments on frequency dependence are either wholly or partly manipulative and concentrate on color morphisms. Epperson and Clegg (1987) found the rare white morph of *Ipomoea purpurea* to be undervisited (but not the colored morphs) and Gigord et al. (2001) proved negative frequency-dependent selection in the rewardless orchid *Dactylorhiza sambucina*, both supporting the lab experiments. However, Eckhart et al. (2006) was the first to prove negative frequency dependence for a rewarding species (*C. xantiana* ssp. *xantiana*). Other studies had no significant results (eg. Jones 1996; Mogford 1978). Experiments on natural flower communities lack completely to our knowledge.

While foraging models are comparatively common, few investigate frequency dependence. The game-theoretic model by Kunin and Iwasa (1996) suggests pollinators should favor common flower types over rarer ones when resources availability is high. The similar mathematical model of Song and Feldman (2014) also concentrates on the pollinator perspective by applying rules of optimal foraging strategy and observe under which conditions the pollinators are able to maximize their net energy intake. Spatial explicit models grew in number over the last years addressing a range of foraging topics (Dornhaus et al., 2006; Bukovac et al., 2013; Faruq et al., 2013). Frequency dependent pollination is only subject to the model by Hanoteaux et al. (2013) who tested survival strategies for less attractive species over multiple generations.

Gap in knowledge

Previous research on FD is scarce and inconsistent between lab, field and simulation data. All field experiments were partly or fully manipulated studies on color morphs. Eckhart et al. (2006) was the first to study a rewarding species and to include natural frequencies. Still, rewarding flowers are underrepresented and studies of natural flower communities lack completely. Furthermore, direct comparison of model and field data to cross-validate findings were only done for related questions such as density effects (Essenberg, 2012) and the learning abilities of bees (Dyer et al., 2014) but never for FD.

Next to the yet not fully proven existence of FD are its influencing factors which can be responsible for differing results of previous research. Smithson (2001) hypothesized in her review about possible reasons but until now, no study was conducted to fill the knowledge gap.

Floral cover is known to influence the foraging behavior of pollinators (eg. Kunin 1993; Essenberg 2012). However, a possible interaction with frequency dependence was not considered in most cases. Exceptions were

Smithson and Macnair (1997) who observed visitation rates for densities between 5 an 10% in their lab experiment without any significant result and Kunin and Iwasa (1996) and Song and Feldman (2014) who included density as factor in their mathematical model. Field experiments generally lack cover analysis.

In contrast to habitat fragmentation, the influence of spatial structure and distribution of flowers is not well studied. But flowers typically exist in patchy distributions of various sizes. Usually, the proportion of flowers visited by pollinators decline with increasing cluster size, probably due to limited memory structure and the avoidance of previously visited flowers (Goulson, 2000). Geslin et al. (2014) found the foraging behavior of bumble bees (*Bombus terrestris*) affected by the spatial distribution of two co-flowering species in a controlled lab experiment. Again, the only study about spatial distribution of flowers in the context of frequency dependence was done by Hanoteaux et al. (2013). Within their model four levels of flower agglomeration significantly influenced the survival rate of the less attractive species (best survival rates: high cluster for low frequencies, low cluster for high frequencies).

My approach/Questions

Given the general low quantity of studies concerning frequency dependent pollination and their inconsistent results I want to address the following questions this thesis:

1. Does frequency dependent pollinator foraging exist for rewarding species in natural floral communities?
2. What kind of a frequency dependent relationship can be found?
3. What are important factors influencing frequency dependence?

I collected data on per-flower visitation rates of five different flowering rewarding plant species. Observations were made over a range of natural frequencies in their grassland plant communities on the area of the Jena Experiment. To explore the important factors influencing frequency dependence I developed a spatially explicit agent-based model of two rewarding co-flowering plant species sharing pollination services. Agent-Based Models ("ABM", also known as Individual-Based Models "IBM") are a valuable tool for assessing interactions in dynamic networks like financial markets, game theory, spread of diseases or, like this case, ecosystems (DeAngelis and Mooij, 2005). The model contains multiple agents which behave independently after given behavior rules and are able to interact with the environment and each other. Agent-based models are especially suitable for analyzing behavior shifts with changing environmental conditions like frequency, floral cover and cluster size. Subsequently, the results of the model were compared to field data to understand influencing factors for FD.

2 Field Study

2.1 Methods

Study Site

The data used in this analysis were collected in the Jena Experiment, located north of the city of Jena in the centre of Germany ($N50^{\circ}55' E11^{\circ}35'$; 130 m a.s.l.) in July and August 2014. Mean annual temperature is 9.3°C and mean annual precipitation 578mm (Kluge et al., 2000). In 2002, 10ha of strongly fertilized arable field in a floodplain of the Saale river were converted into a biodiversity experiment. Species mixes of 1, 2, 4, 6, 8, 16 and 60 species from a pool of 60 common European grassland species were sown in 82 plots à a $20\text{m} \times 20\text{m}$ (Roscher et al., 2004). The Jena Experiment has the purpose to explore the effect of plant diversity (species richness and functional group richness) in grassland communities and is used in numerous studies and experiments.

The plots of the Jena Experiment are mowed twice a year in accord to standard grassland management. Parts of each plot are additionally weeded twice a year to maintain the original plant composition. Two subplots per plot were excluded from the weeding since 2002 ("Old Invasion Plots", $4\text{m} \times 5.5\text{m}$, 22m^2) and since 2009 ("New Invasion Plots", $5\text{m} \times 3.5\text{m}$, 17.5m^2) to evaluate invasive potential and effects. In 2014, subplots with continuous weeding were scarce with flowers and had a generally low species richness. Hence I collected the data in the old and new invasion plots with a higher cover, species richness and diversity. From the 82 plots of the Jena Experiment I only included plots with a floral cover between 20% and 70% for better comparability. In total, 23 plots were sampled throughout this study.

Data Collection

I selected the focal plant species during the field work as the flora changed very quickly and unpredictably. A focal species had to be flowering for at least one week in the sampling time and be present in at least five plots with a differing frequency to get sufficient data. Therefore, I chose *Lathyrus pratensis*, *Lotus corniculatus*, *Trifolium pratense* and *Onobrychis viciifolia* of the family Fabaceae and *Geranium pratense* of the family Geraniaceae (Supplementary material, tab. S1).

Pollinator observations were only made during suitable weather conditions (at most partly overcast, no more than light wind, min. 15°C). The sampling took place between 9am and 5pm. Overall, 15 days between 20th of July and 12th of August 2014 were suitable for pollinator observations. During each observation bout I recorded all pollinator activity during 15 minutes in a patch of $80\text{cm} \times 80\text{cm}$. This size is feasible to watch even with high pollinator activity and floral cover. The data collection included all visits to flowers of the focal plant species and total visitation number for all other flowers in the patch. I counted the flowers of the focal species to calculate the per-flower visitation rate. As possible drivers for visitation rate changes, I estimated the floral cover and identified all other flowering plant species present on patch and plot level. Each plot contained eight evenly distributed patches for 2h observation time per focal species and frequency.

Statistical Analysis

Per-flower visitation rate was used as response variable to identify frequency dependence. The variable equals the count of visits of all pollinator types to all flowers of the focal species per patch within 15 minutes observation time divided through the number of flowers of the focal species within the patch. Therefore, the response variable is not a count data and was modeled with a Gaussian error distribution in the analysis. The explanatory variables of the full model were measured at plot level and included species richness, floral cover as single terms and frequency as single, quadratic and cubic term. Included interaction were frequency (single and quadratic) with floral cover and species richness as well as frequency (single, quadratic and cubic) and cover in interaction with species. Species was included as nominal response variable. All statistical analysis were performed with R, version 3.1.2. (R Core Team, 2014).

I used variance inflation factors (VIF) to check whether any variables in the dataset are collinear and should be removed prior to the analysis. With all values below two, there was no sign for collinearity and therefore

acceptable to use them in the model selection as explanatory variables (Zuur et al. 2007, supplementary material, tab. S2). Pairwise scatterplots with included correlation of coefficients also showed only minor correlation (Supplementary material, fig. S2).

The sampling design contained 8 observations per plot summing up to 2h of observations per species and frequency. Therefore, the data are not independent and I chose a linear mixed effect model with subplot nested in plot as random effect. I used the function "lme" from the R package "nlme" (Pinheiro et al., 2014) for all further analysis.

The beyond optimal model with the full set of reasonable predictors and interactions showed a strong pattern of heteroscedasticity in the residuals. With the varIdent-function from the R-package "nlme", every species is allowed to have its own variance structure and we can maintain the differences in attractiveness of the five focal species in the model as biological information. The weighting provided a significantly better variance structure for the model ($L = 383.74$, $df = 4$, $p < 0.0001$).

I performed a backward stepwise deletion of interactions and predictors with maximum likelihood estimation (ML) for each model. The loss of explanatory power in the model after removal of a variable was tested by comparing the Akaike information criterion (AIC) of the model with and without the explanatory variable (ANOVA model comparison). If there was no significant loss of explanatory power, the variable was removed. The selection was verified by a global selection via the dredge-function from the R-package "MuMin" (Barton, 2014) with maximum likelihood estimation.

The final model was again validated by plotting the normalized residuals against fitted values. The vertical gap in the residuals can be explained by the difference in flower attractiveness (supplementary material fig. S3). *Geranium pratense* and *Onobrychis viciifolia* received very high visitation rates whereas *Trifolium pratense*, *Lotus corniculatus* and *Lathyrus pratensis* had generally only few visits. However, the heteroscedasticity of residuals could be dealt with by the weighting and the mean of the residuals is close to zero (< 0.0001).

2.2 Results

Visitation Rates

In total, I made 385 observations, each representing pollinator activity records for 15min in a 80cm x 80cm plot. In total, I analyzed data from 96,25h of observation on 246.4m².

Onobrychis viciifolia was the most attractive plant with a maximum of 318 visits in one observation. The per-flower visitation rate varied strongly with the attractiveness of the focal species. Per observation, I recorded 1.4 ± 1.8 (mean \pm SD) visits per flower with a maximum of 10.7 visits per flower (again *Onobrychis viciifolia*) and 31 observation with no visit at all to the focal species. The per-flower visitation rate was significantly different between the two very attractive species *Geranium pratense* and *Onobrychis viciifolia* and the three less attractive species *Trifolium pratense*, *Lotus corniculatus* and *Lathyrus pratensis* ($P \leq 0.001$, tab. 1). The subplots contained 3 ± 1.2 (mean \pm SD) flowering species including the focal species, the species richness was higher at the plot level with 8 ± 2.4 (mean \pm SD) flowering species.

Table 1: Per-flower visitation rates (mean \pm SD) for the focal flower species per 15 minute observation. *Geranium pratense* and *Onobrychis viciifolia* are significantly different from the other three species (pairwise t-test, $p < 0.001$). Within the two groups, there is no significant difference between the species.

Short	Species	Family	Visitation Rate (Mean)	\pm SD
Ger	<i>Geranium pratense</i>	Geraniaceae	3.05	1.5
Lat	<i>Lathyrus pratensis</i>	Fabaceae	0.57	0.53
Lot	<i>Lotus corniculatus</i>	Fabaceae	0.30	0.36
Ono	<i>Onobrychis viciifolia</i>	Fabaceae	3.60	2.5
TP	<i>Trifolium pratense</i>	Fabaceae	0.16	0.23

Frequency Dependence

Floral cover and species richness had both individually and in the interaction term with frequency no effect on the visitation rate and were removed from the model (Cover: $F_{df=1} = 1.17$, $P = 0.28$; Species Richness: $F_{df=1} = 1.15$, $P = 0.29$).

The linear mixed effect model showed an effect of species and frequency individually and with interactions on the per-flower visitation rate (Species: $F_{df=4} = 141.13$, $P \leq 0.0001$; Frequency: $F_{df=1} = 18.29$, $P \leq 0.0001$; Species x Frequency $F_{df=4} = 5.2$, $P \leq 0.001$, tab. 2). Interestingly, frequency contributed also as quadratic and cubic term with its interactions to species explanatory power to the model, giving the relationship a non-linear character (Tab. 2). Figure 1 showed the cubic relationship of four focal species and the summed data and a quadratic relationship for *Lathyrus pratensis*. The cubic curve is defined by a strong increase for frequencies below 20% followed by a minimum between 50 and 80% depending on the species before raising again with increasing dominance of the focal species. However, the visitation rate of *Lathyrus pratensis* showed a maximum at 60% frequency and decreases afterwards.

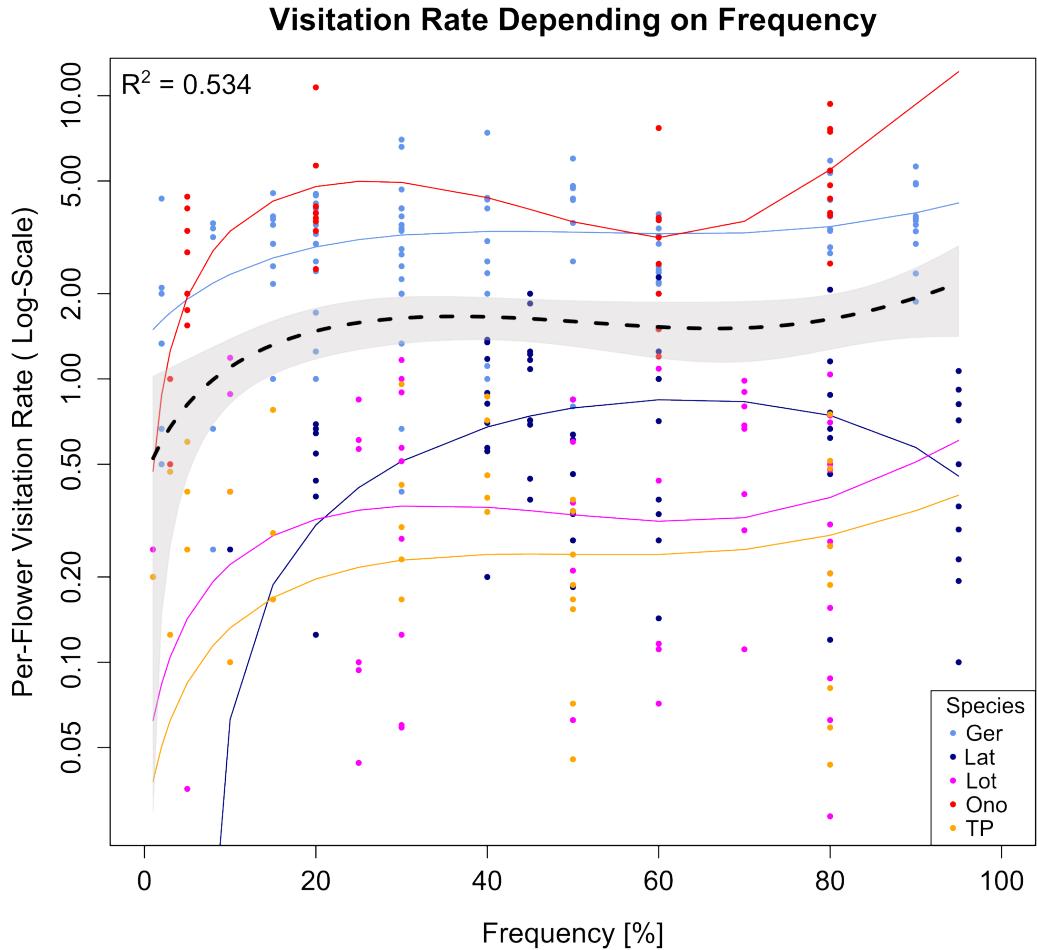


Figure 1: Per-flower visitation rates of the five focal species over different frequencies. Each point represents one observation of 15 minutes. The y-axis is plotted on a log scale due to the variance in attractiveness of the focal species. The linear mixed effect model with patch nested in plot as random factor show a cubic frequency dependence for all species but *Lathyrus pratensis*. Floral cover and species richness were dropped as explanatory variables in the model selection process. R^2 was calculated with the "r.squaredGLMM"-function of the MuMIn-Package (Barton, 2014)

3 Agent-Based Foraging Model

3.1 Methods

Main Features

The model was developed based on empirical findings for foraging rules and pollinator behavior. It is a simple spatial model of two co-flowering plant species competing over pollination service.

In the model, all pollinators (from now on called "bee-agents") are identical and the two flower types only differ in their species identity. Reward replenishment, handling times to extract the reward and its attractiveness towards the bee-agents is identical for both species. Corolla color is only assigned for better visualization and is not important for the model or the bee-agents, respectively. All bee-agents behave under the hypothesis of flower constancy which is empirically tested for various pollinators (e.g. HILL et al. (1997) for honey bees, Chittka et al. (1997) for bumble bees, Goulson and Wright (1998) for hoverflies and Goulson et al. (1997) for the butterfly

Table 2: Results of the linear mixed effect model with per-flower visitation rate as explanatory variable. Floral cover and species richness and its interactions with frequency were not relevant predictors for the model and therefore removed in the model selection process (denDF = 191, $R^2 = 0.53$, n = 385)

Response Variable	Explanatory Variables	Df	F-value	P
Per-flower visitation rate	Species	4	130.9	< 0.0001
	Frequency	1	49.3	< 0.0001
	Frequency ²	1	13.2	0.0026
	Frequency ³	1	5.8	0.8145
	Frequency x Species	4	5.2	0.0005
	Frequency ² x Species	4	3.4	0.0097
	Frequency ³ x Species	4	3.4	0.0101

Thymelicus flavus). Flower constancy is the tendency of an individual pollinator to keep visiting the same flower species instead of switching to more rewarding or closer species (Chittka et al., 1999; Waser, 1986). Because we are interested in the visitation rate of flowers in different frequencies, the energetic costs and the limit of gained rewards of the bee-agents are ignored. Furthermore, they do not communicate and always empty a flower completely.

Model Environment

I used NetLogo (Wilensky, 1999) as programming environment. It is a simple but powerful tool for making ABMs and connectible with R through the R-package "RNetLogo" (Thiele et al., 2012). In NetLogo, the "world" is a spatial grid with a set number of cells called patches. Agents can move freely over the patches according to their given behavior rules. Patches and agents both have own properties and can interact with each other. In my model, the "meadow" has 100x100 grid cells with horizontally and vertically wrapping to avoid edge effects. Every grid cell can either contain a single flower of one of the two species or grass.

Figure 2 shows a set of exemplary model environments. Floral cover is defined as the percentage of the patches containing flowers and the cluster number equals the average number of flowers within a cluster. The number of clusters per species can be calculated by dividing the number of flowers per species on the meadow through the cluster number. If the cluster number is one, all flowers are randomly distributed over the meadow. With increasing cluster size, one flower per cluster is assigned on the meadow ("cluster-seeds") and all remaining flowers of that species are randomly allocated to them in a second step.

Every flower contains 1 Joule of reward in the beginning of each simulation run. The bee-agents are randomly distributed over the modeling environment and start without a fixed preference for a flower type but just pick the closest one when the simulation starts. Every time step in NetLogo equals one second.

Behavior Rules

All bee-agents act independently from each other according to the behavior rules shown in Figure 3 (Overview of all parameters used for the model with its default settings in the supplementary material in tab. S4).

As mentioned in the assumptions, the behavior of the bee-agents is strongly influenced by flower constancy (e.g. Bobisud and Neuhäus, 1975; Chittka et al., 1997; Thomson, 1981; Chittka et al., 1999; Goulson, 1994, 1999). Bee-agents always prefer one of the two flowering species and forage exclusively on this species. The preference can change due to lack of searching success or a series of low rewards of the preferred flower (Chittka et al., 1997; Kunin, 1993; Greggers and Menzel, 1993). At any given time, a bee-agent can be in one of two states: search for a flower or visit one.

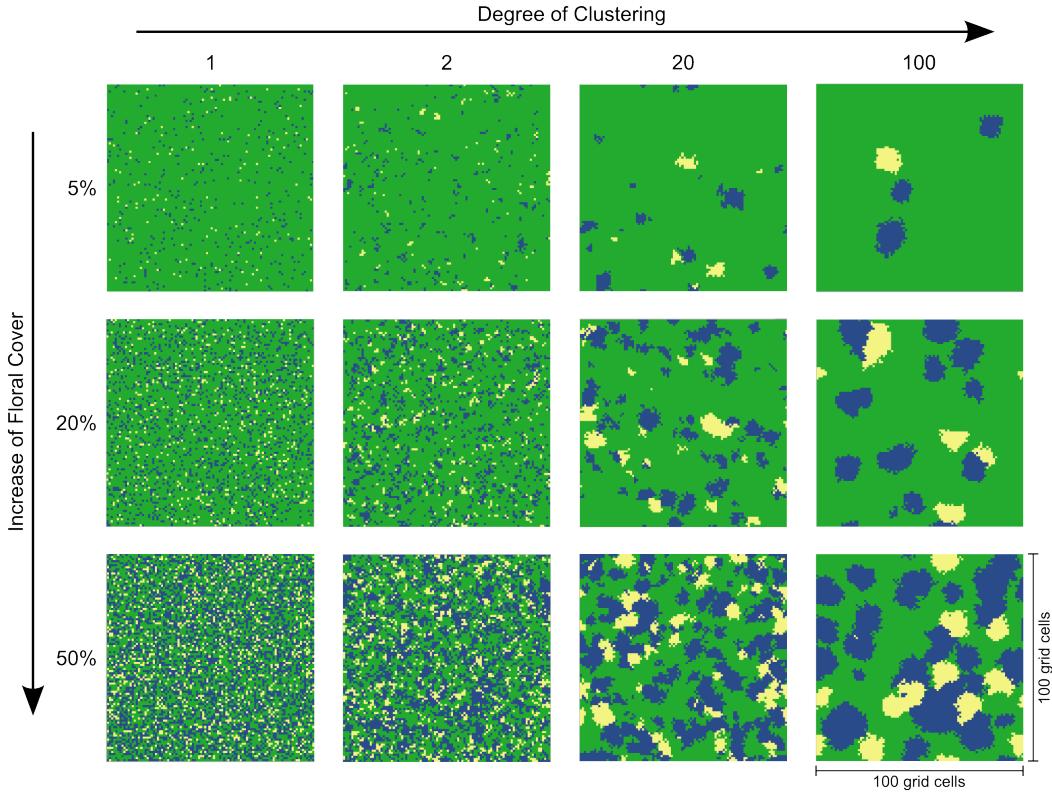


Figure 2: Exemplary model environment setups with increasing floral cover and cluster size. The cover expresses the percentage of patches containing a flower ($\Sigma_{\text{patches}} = 10\,000$). The cluster number equals the average amount of flowers per cluster. Flowers are randomly assigned to the clusters in the model setup.

Search

Pollinators avoid recently visited flowers (Goulson, 1999). Every bee-agent possesses with a memory to remember the location of the last four already visited flowers (Goulson, 2000). If there is any not recently visited and preferred flower in sight, the searching bee-agent moves directly towards the flower, otherwise it continues searching.

Previous research on the speed of foraging pollinators by Essenberg (2012) and Kunin (1991) (in Kunin and Iwasa 1996) gives 0.1m/sec as benchmark. Consequently, bee-agents can move 1 grid cell per time step in this model. The vision of pollinators was studied in various experiments using a Y-maze apparatus (Dyer et al., 2008; Wertlen et al., 2008; Ne’eman and Kevan, 2001). Every bee-agent can detect flowers from a distance of 0.7m with an equivalent of 6 grid cells. The vision is reduced to a 180° cone-shaped field to the front of the agent. Pollinators tend to keep their direction while foraging (Waddington, 1980). In the model, I used a correlated random walk (CRW) to achieve a relatively natural movement (Bartumeus et al., 2005; Codling et al., 2008; Pyke and Cartar, 1992; Viswanathan et al., 2008). Empirical studies have shown an increasing probability to abandon the original flower preference the longer the search remains unsuccessful (Chittka et al., 1997; Kunin, 1993). If the bee-agent searches for 5 seconds (= 5 time steps) without finding any preferred and unvisited flower, the likelihood of changing its preference increases by 10% with every additional time step.

Visit and Reward Intake

When a bee-agent encounters a preferred and unvisited flower it takes up all its reward. As long as the reward content of a flower lower than the maximum it is refilled by 0.00004 Joule in each time step until the maximum of

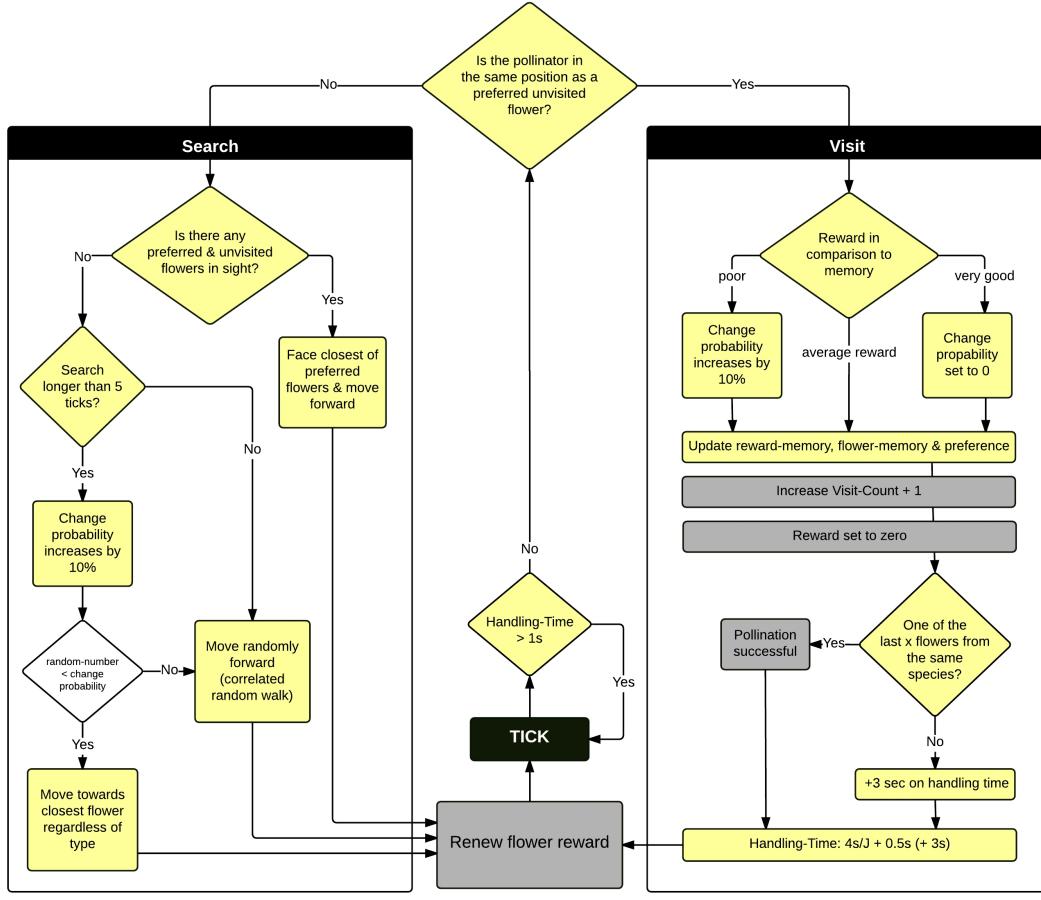


Figure 3: Flowchart describing the behavior rules for the bee-agents within the agent-based model. At any time given, a bee-agent can either search for a preferred flower or visit one. While searching, a bee-agent can remember the location of the last four visited flowers to avoid double-encountering. If there is no flower in sight after 5 seconds of correlated random walk (CRW), the probability that it will encounter the next available flower despite its type increases by 10% per additional time step. When a bee-agent visits a flower it takes all reward within a reward-dependent handling time and compares the amount with its memory. If the reward is low, the agent is more likely to visit the other flower type next time. The maximum of visits within a successful pollination is possible (x) is determined by the pollen carryover parameter.

1 Joule is reached (see "reward-function" in tab. S4). The handling time of a bee-agent visiting a flower involves three components: a time proportional to the amount of reward taken, a reward-independent constant and a skill factor (Kunin and Iwasa, 1996). In my model, a bee-agent requires 4 seconds to extract one Joule of reward plus a reward-independent handling time of 0.5 seconds. When the bee-agent just changed its flower preference it gets a 3 second penalty for inexperience (Roubik 1992, Kunin and Iwasa 1996).

The reward taken is stored in each individual's reward-memory. Every agent can remember the last four rewards received. When visiting a flower, the bee-agent compares this memory with the current reward quantity. If the reward is less than half the average in the memory, the likelihood to abandon flower constancy and visit the other species next increases by 10%. If the reward is exceptionally good (at least twice the remembered average), the change probability is set to zero (Chittka et al., 1997; Keasar et al., 1996).

Additional to the quantity of visits we're also interested in their quality. A flower can only be successfully pollinated if the bee-agent visited the same species before. The maximal number of heterospecific flower visits which still allows successful pollination is determined by the degree of pollen carryover. The parameter applies

to all bee-agents and can have a value between 1 and 16. For the value of one, the very last visit has to be from the same species (strong heterospecific pollen interference, see Campbell 1986; Benadi et al. 2012; Montgomery 2009).

After reward-collection is completed, the bee-agent updates its flower-memory and its reward-memory and continues foraging. Each visit and successful pollination is recorded for later analysis.

Simulation experiments

Parameters altered in the main analysis are frequency, floral cover, degree of clustering and pollen-carryover. Each parameter-combination was run 20 times with a length of 1000 ticks each (110,400 runs in total). Additionally, I performed a sensitivity analysis with additional parameters which affect the behavior of the bee-agents to understand how the values of these parameters influence the frequency dependence of per-flower visitation rate. Table 3 presents the definition and value range of the parameters.

Table 3: Parameter values used for the main and sensitivity analysis. Only general parameters were changed in the main analysis, whereas the sensitivity analysis also directly influences the behavior of the bee-agents. Each combination was repeated 20 times for 1000 time steps, that makes a total of 110,400 runs in the main analysis and 16,560 runs for each parameter of the sensitivity analysis.

Parameter	Description	Values
MAIN ANALYSIS		
Frequency	Proportion of species A on all flowers	0-100% (5%-steps)
Flower cover	Proportion of patches containing a flowers	5, 10, 20, 50 %
Degree of clustering	Average number of flowers per cluster	1, 2, 5, 10, 20, 50, 75, 100
Pollen carryover	Number of visits within a successful pollination is possible	1, 2, 4, 6, 8, 16
SENSITIVITY ANALYSIS		
Reward function	Increase of reward per flower and second	0, 0.00004, 0.001, 0.1 J/sec
Vision distance	Range of patches within a bee-agent can detect flowers	1, 6, 20, 50 patches
Search time	Number of seconds a bee-agent searches before the probability of switching flowers increases	1, 5, 20, 50 sec
Pollinator density	Number of bee-agents on the meadow	5, 10, 20, 50 bees

3.2 Results

Per-flower visitation rate

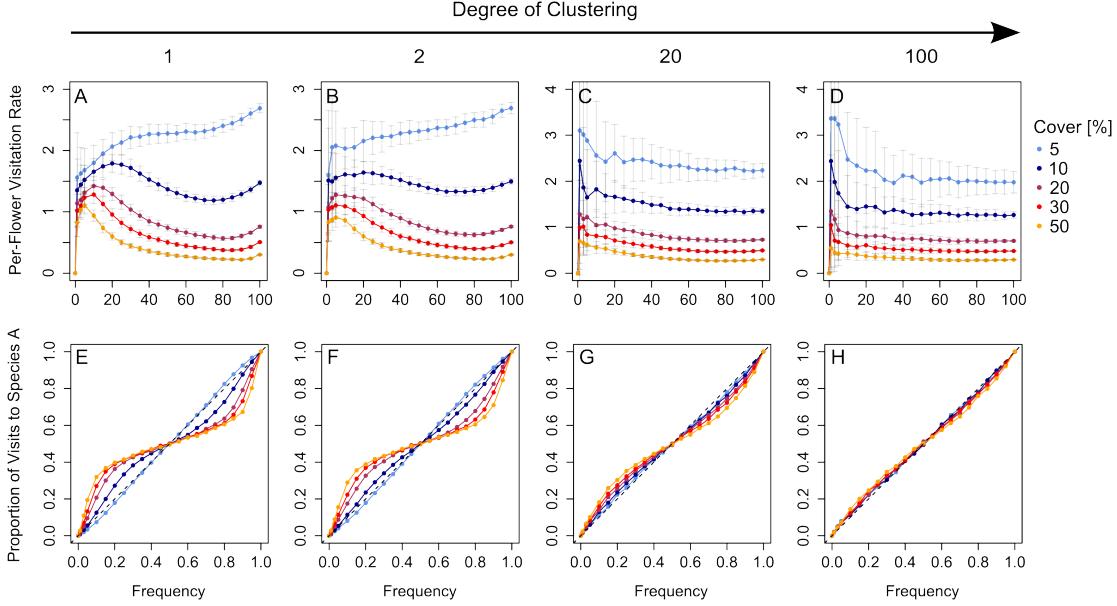


Figure 4: The main analysis of the agent-based model confirms the findings of the linear mixed effect model of the field data: The per-flower visitation rate shows a frequency dependence with a cubic relationship. Visitation rates increase within the first 10–20% of frequency towards a maximum. Afterwards, the additional gain of visits is not proportional with the increase of flowers due to higher frequency. The per-flower visitation rate only increases again towards exclusiveness of the species. The effect is stronger for higher floral cover (2a-d). Increase in clustering reduces the frequency dependence (1d,2d).

The per-flower visitation shows a similar cubic function as the data collected in the Jena Experiment (Fig. 4A,B). Within the first 20% there is a steep increase in visits per flower. Afterwards, since for all cover values above 5% the additional gain of visits is not proportional to the increase of flowers due to higher frequency, the per-flower visitation drops with a minimum around 80%. Close to 100%, when the species becomes dominant, the per-flower visitation rises again. Cover and cluster size both influence the frequency dependence. The higher the cover, the lower the per-flower visitation and the bigger the clusters the less visible is the frequency dependence. Simulations with more than 10 flowers per cluster show a high variance for frequencies below 10% and very low to no frequency dependence afterwards (Fig. 4C,D).

The same data is plotted as proportion of visits to species A to take the variance in the sum of visits into account (Fig. 4E-H; see section "Global visitation"). It shows a clear negative frequency dependence favoring the rare species. Below 50%, species A receives more visits than would be proportional, above 50% the curve is mirrored because both species are identical in the simulation and the common species gets disproportional few visits. The higher the cover, the stronger is the frequency dependence. For higher cluster values, all data points approach a frequency independent relationship (Fig. 4H).

Pollination success

The degree of pollen carryover is defined as maximum number of heterospecific visits within a successful pollination can take place. In the model, I tested values from 1 (strong heterospecific pollen interference) to 16 (weak heterospecific pollen interference). Figure 5 gives the proportion of all visits where a successful pollination took place. The first 20% frequency are crucial for all parameter-value combination. A very steep increase up to 80%

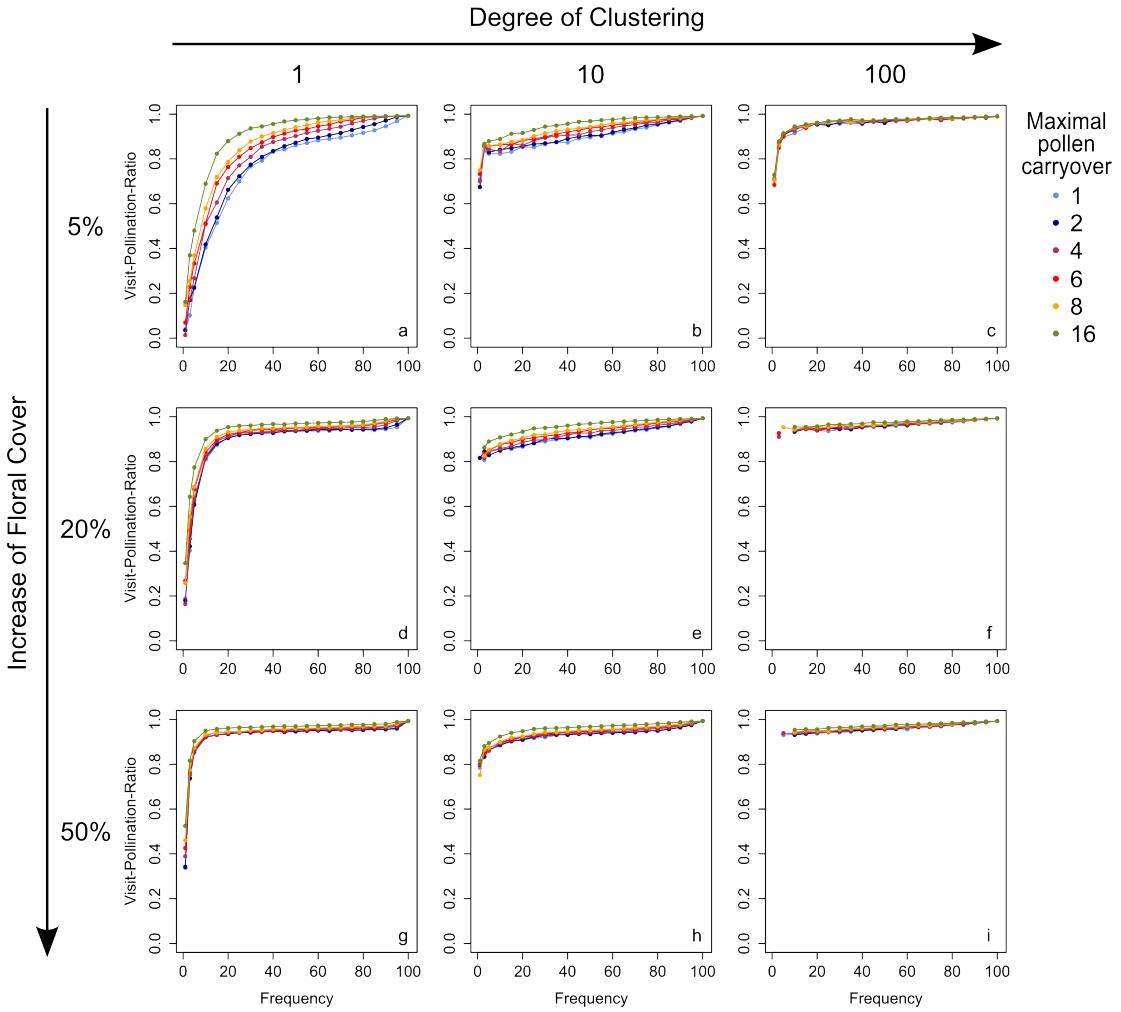


Figure 5: The pollen-carryover rate defines the maximum number of visits within a successful pollination is possible. With a pollen-carryover rate of one, the pollen can only be carried to the next flower. Therefore, the ratio of successful pollinations per visit can be seen as indicator for flower constancy (Montgomery, 2009). A high pollen-carryover rate is only important for a low cover and no-cluster environment. With increasing cover and cluster, the ratio becomes steeper for low frequencies which stands for more qualitative visits.

successful pollinated flowers is followed by a moderate linear increase up to 100% for exclusive existence. The degree of pollen carryover only makes a difference for small cover and cluster values (Fig. 5a). The higher the cover and the bigger the clusters, the better is also the proportion of successful pollination, even for small frequencies, independent of the pollen-carryover rate (Fig. 5c,f,g-i).

Global visitation

The sum of all visits to both plants together changes with the ratio of their abundance (Fig. 6). A strong cover-dependent pattern is visible for small cluster sizes. The total visits have a u-shaped relationship with frequency for 5% cover and a shape similar to a fourth-degree polynomial function for higher cover values. The visitation drops to a minimum at 90:10 ratio and peaks again for balanced frequencies. Cluster reduces the frequency dependence.

Note that the mean number of total visits varies in addition to the shape for different values of cover and cluster size. Both parameters have a frequency independent influence on the visitation rate (supplementary ma-

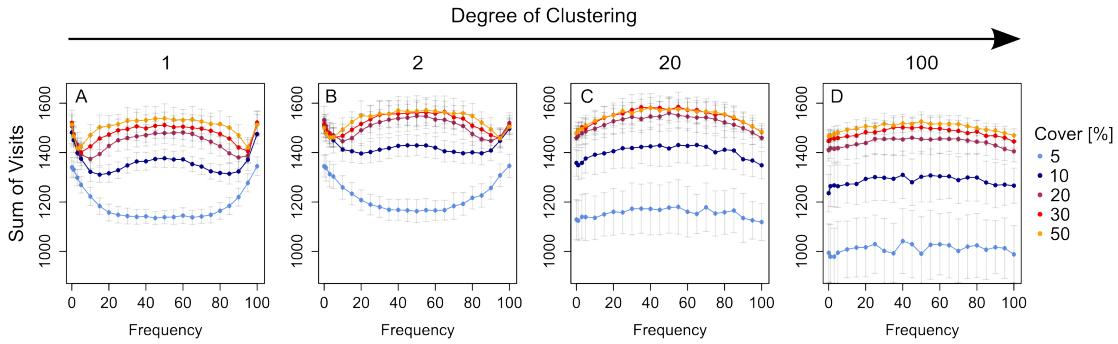


Figure 6: Summed visits to both species show a frequency dependence for low cluster values. Depending on the floral cover it is quadratic or a fourth-degree polynomial relationship. Maximum of visits (maximal efficiency) is achieved for very unequal or very equal frequencies. If one species is rare, the sum of visits drop because some bee-agents forage inefficiently on the rare species, having long flight and searching times. Clustering reduces the frequency dependence but also decreases the absolute number of visits and increases the variance (grey error bars).

terial, fig. S4). Floral cover shows a saturated curve and the visits for degree of clustering have a hump-shaped relationship with a peak at an intermediate aggregation level of 5-10 flowers per cluster.

Sensitivity analysis

Aim of the sensitivity analysis is to understand influence of behavioral rules on the outcome of the model. Therefore, pollinator density, reward regrowth, search time and vision were tested for a (unnaturally) broad range of values set in comparison to the empirically founded default values. Vision, search time and the number of bees on the meadow influence the sum of visits (Fig. S5). A larger range of vision leads to more visits, the search limit reduces the number of visits and more bees lead again to more visits per time unit. The reward function has an small negative influence on the total number of visits for a very high regrowth rate.

Furthermore, a very high replenishment rate (0.1J/sec, yellow line in fig. S6) has a reversing effect on the frequency dependence: Rare species receives disproportionately few visits whereas common species benefit from a positive frequency dependence. The influence of frequency is less severe with increasing cluster size.

Every bee-agent can detect flowers in a 180° cone-shaped array of patches in front of them. The number of patches in that array is determined by the vision distance. A large range of vision increases the frequency dependence even in a heavily clustered model environment (Fig. S7). If the bee-agents are only able to see the direct neighbor, the frequency dependence is reversed to favor the common species for low cluster values.

If a bee-agent searches longer than a given search time limit unsuccessfully for a unvisited and preferred flower, the probability to switch preferences will increase by 10% with every additional step. The search limit was altered from 1 to 50 seconds in the sensitivity analysis. The results are similar to the effect of vision as they also change the probability to switch preferences. Higher search time limits lead to stronger negative frequency dependence benefiting the rare species. A search limit of 1 second reduces the dependency (Fig. S8).

Beside the expected increase of absolute visits, a change of pollinator density has no effect on the outcome of the model at any cover or cluster values (Fig. S9).

4 Discussion

Frequency dependence can have far reaching consequences for the development and maintaining of biodiversity. Aim of this thesis is to study the existence of frequency dependence in a natural plant community, explore the kind of relationship and understand the underlying rules and drivers for frequency dependence with the help of an agent-based model. The results of the natural condition data are consistent with the simulation data: A distinct frequency dependence within the per-flower visitation rate (cf. fig. 1 and fig. 4A). The relationship is defined by a steep increase of visits within the first 20% frequency followed by a disproportional low gain of visits for every additional flower due to an increase of frequency. When the species becomes dominant the per-flower visitation rate increases again. Additional simulations confirm the negative frequency dependence as outcome of the empirical based default values.

Explaining negative frequency dependence

Previous research found positive FD for lab experiments and inconsistency in the few field experiments focusing on color morphs (review by Smithson 2001). However, the field data suggests a negative FD for at least four different rewarding flower species confirmed by the results from the foraging simulation. Where does the discrepancy of lab and field data comes from?

The sensitivity analysis of the ABM can give an explanation: If the reward function is increased to a refill within 10 time steps, the relationship is reversed to a positive frequency dependence and the rare species receives disproportionately few visits (Fig. S6). The curve is highly consistent with findings of Smithson and Macnair (1997) and Smithson and Macnair (1996) in their lab experiments. In their study design, artificial flowers were refilled after each foraging bout. Therefore, every bumble bee foraged on a set of full and equally rewarding flowers which is comparable to a high regrowth function in the ABM.

We know that pollinators more likely abandon flower constancy if they experience sequentially bad reward (Chittka et al., 1997; Goulson, 1994). If the reward is always high, pollinators have less incentive to go on exploratory visits to the rare species as the abundant type is easy to find and sufficient rewarding. Hence it can be assumed that negative frequency dependent selection does not exclusively apply for non-rewarding species but also for flowering communities with varying or insufficient reward. Positive frequency dependence in pollination might be only possible for highly rewarding or artificial systems. If negative frequency dependence is in fact found for a variety of rewarding flowers, I agree with Eckhart et al. (2006) that frequency dependence might be more important in the development and conservation of diversity than previously recognized. Also, positive and negative frequency dependence might not be object to certain species but occur spatial and time related.

Cover and Cluster size are influencing factors

The model reveals two main influences for frequency dependence: The higher the floral cover, the stronger the frequency dependence and the bigger the clusters, the lower the frequency dependence (Fig. 4E-H). Floral density is known to influence visitation rates, usually positive and with a saturating function (e.g. Rathcke 1983, Essenberg 2012, Bernhardt et al. 2008, Kunin 1997). Those findings are consistent with the Hollings type II functional response found for different cover values (Supplementary material, fig. S4a). If the cover is increasing, the absolute number of flowers rises also for the rare species. That makes it more likely for a bee-agent to find a flower before changing preference towards the common species even if foraging on the later would be more efficient. Therefore, high cover causes the same effect as expanded vision distance or maximum search limit (cf. fig. S7 and fig. S8): The main reason of abandoning flower constancy becomes multiple visit of flowers with low reward. The following explanatory visits to the rare species can have a great impact on the per-flower visitation rate. Every visit to a rare species weights high in the per-flower visitation rate because the sum of visits is divided through the number of flowers.

The model shows that spatial aggregation of flowers can lead to a more efficient foraging (more visits per time unit), less FD and a higher quality of visits due to compatible pollen deposits. If flowers are random and even distributed, many short search and flight times apply. An intermediate cluster level is easy to exploit by a pollinator whereas the flight and search times can be very long in between few big clusters, especially for low floral densities (Supplementary material, fig. S4b). It was already suggested by Epperson and Clegg (1987) that

spatial agglomeration of flowers decreases frequency dependence. In the ABM, a similar effect compared to low cover takes place: If flowers are aggregated at few places, they affect the pollinators perception of frequency and are more difficult to find. Long search times will weaken the bee-agents flower preference and lead to foraging on the next available cluster independent of its containing species.

Requirements for successful pollination

High visitation rate is gained at low frequency with high cover and low cluster size. However, those visits might not be the best quality if the pollination per visit ratio is comparatively low (Fig. 5a,d). The ratio can be seen as index for flower constancy: If the majority of visits lead even for a small pollen-carryover value to a successful pollination the bee-agents perform flower constancy (Montgomery, 2009). If the cover is high, bee-agents will keep their constancy also for rare species because they are abundant enough. If the aggregation of flowers is high, bee-agents exploit this cluster before leaving for the next. Every visit within a cluster of flowers of the same species is counted as successful pollination and can lead to a high visit quality even if the cover is low (cf. Jakobsson et al. 2009)

Therefore optimal pollination is reached by rare species to stand in clusters of flowers if the cover is low. If the cover is high the spatial distribution plays a minor role for the visit quality.

Frequency dependent sum of visits to the flower community

Additionally to individual frequency dependence, I analyzed the impact of species partitioning on frequency dependent visitation in the system as whole.

If the cover is very low, a maximum in total visits can be received if one species is dominant. Co-flowering will lead to longer search times and less overall visits (u-shape for 5% cover in fig. 6a). For higher cover, the frequency dependence shows a function similar to a fourth-degree polynomial. If one species is rare at 5-20%, some bee-agents have at least exploratory visits to the rare species and spend inefficient time searching. Hence, the total visitation number drops to a minimum. If species are evenly distributed the pollinators forage on both species in equal amounts. This is the most efficient status for the overall ecosystem, especially for high covers or cluster size.

Spatial aggregation weakens the frequency effect but will also reduce the total visits. If flowers are evenly and random distributed, the bee-agent has many small search times intermittent by collecting reward on a single flower and continue foraging. Rare flowers can be found comparatively easy if they are spread over the whole meadow and flower constancy will be kept even if it is highly inefficient. If the clusters of flowers are bigger, bee-agents will not find rare flowers that easily because they might occur only in a single cluster on the meadow. The bee-agent will switch to the common flower, the minimum at very uneven distribution disappears and the relationship becomes slightly hump-shaped (Fig. 6b,c).

A maximum of overall visits favoring both the pollinators and the co-flowering plants can be achieved with balanced abundance in high cover and very uneven species distribution for low floral cover environments. These findings could be tested by manipulated field experiments. Natural conditions data are not suitable for this purpose because every plot contains more than two co-flowering species with unequal attractiveness.

Limitations of the study design and research suggestions

Even though modeling can be an excellent tool to understand and interpret ecological data, some questions evolve comparing the data collected in the Jena Experiment and the foraging model. Floral cover is an important factor in the outcome of the model. It influences not only the absolute number of visits but also the intensity of frequency dependence. But it was removed in the model selection as it was no factor of explanatory power to the per-flower visitation data. Reason could lie in the sampling design. Data was only observed from plots with an intermediate cover, no extremes were taken into account. In total, there were only five values for cover in the final analysis. Also all cover values are estimations, no exact measurements. Another drawback are the lack of data for cluster size and pollination success. The experimental design and time restraints made it impossible to take more predictors into account. The data collected in Jena shows drastic differences in attractiveness of the focal species and frequency dependence was found to be subject to each species. Therefore I suggest research on

a variety of species, both rewarding and unrewarding. Validation if further results of the ABM can be made via a supplementary study with varying frequency, cover and cluster values of two co-flowering species. Either under natural conditions where manipulation is possible (eg. Eckhart et al. 2006; Essenberg 2012) or with potted plants (Epperson and Clegg, 1987).

5 Conclusions

In conclusion, this study shows for the first time that frequency dependent selection exists in natural flowering communities for rewarding species. Also, a combination of methods is exceptionally helpful to understand influencing factors. The output of the agent-based foraging model confirms the results of the field data and fills the knowledge gap of previous research: Positive frequency dependence proved multiple times in lab experiments is likely due to very high rewards. Negative frequency dependence is therefore not exclusive to non-rewarding morphs but takes effect also for common rewarding species if the reward is not exceptionally high. Patterns of frequency dependence can therefore change across space and time, especially because the model revealed floral cover as FD-increasing and spatial aggregation of flowers FD-reducing factor.

Those findings are important for our understanding of the evolution and conservation of diversity. Negative FD, thus if rare flowers have an advantage in pollinator visits, might be an important factor in the evolution not only floral polymorphisms but diversity as such.

Further research is necessary to validate the role of floral cover and cluster for FD. A controlled field experiments including measurements of floral reward and pollination success could be a suitable approach. Based on the findings, I also recommend the connection of modeling, field and lab work. Most research is only done in one of those three approaches with a lack of cross-validation. It could be gained a great deal of knowledge by establishing interdisciplinary working groups of field ecologists and environmental modeling experts.

What else? (Nor yet included)

- POC
- Best for ecosystem and bees (most visits, most efficiency): extreme or balanced frequency, high cover, intermediate cluster

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Supplementary Material

Table S1: List of the focal species observed in the natural condition experiment within the Jena Experiment. Species had to flower in at least five plots with different frequency values to be considered as focal species.

Short	Name	German Name	Order	Family	Color
Ger	<i>Geranium pratense</i>	Wiesen-Storzschnabel	Geriales	Geraniaceae	Purple
Lat	<i>Lathyrus pratensis</i>	Wiesen-Platterbse	Fabales	Fabaceae	Yellow
Lot	<i>Lotus corniculatus</i>	Gewöhnliche Hornklee	Fabales	Fabaceae	Yellow
Ono	<i>Onobrychis viciifolia</i>	Saat-Esparsette	Fabales	Fabaceae	pink+white
TP	<i>Trifolium pratense</i>	Wiesen-Klee	Fabales	Fabaceae	Purple

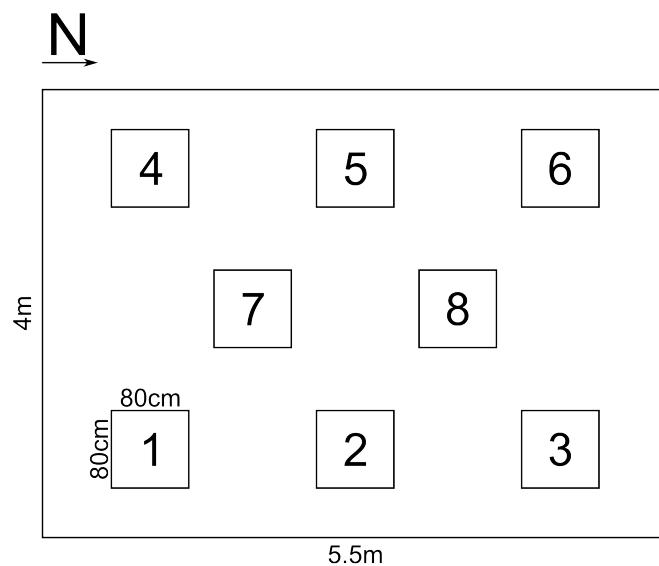


Figure S1: The distribution of subplots within the old invasion plots.

Collinearity of Variables

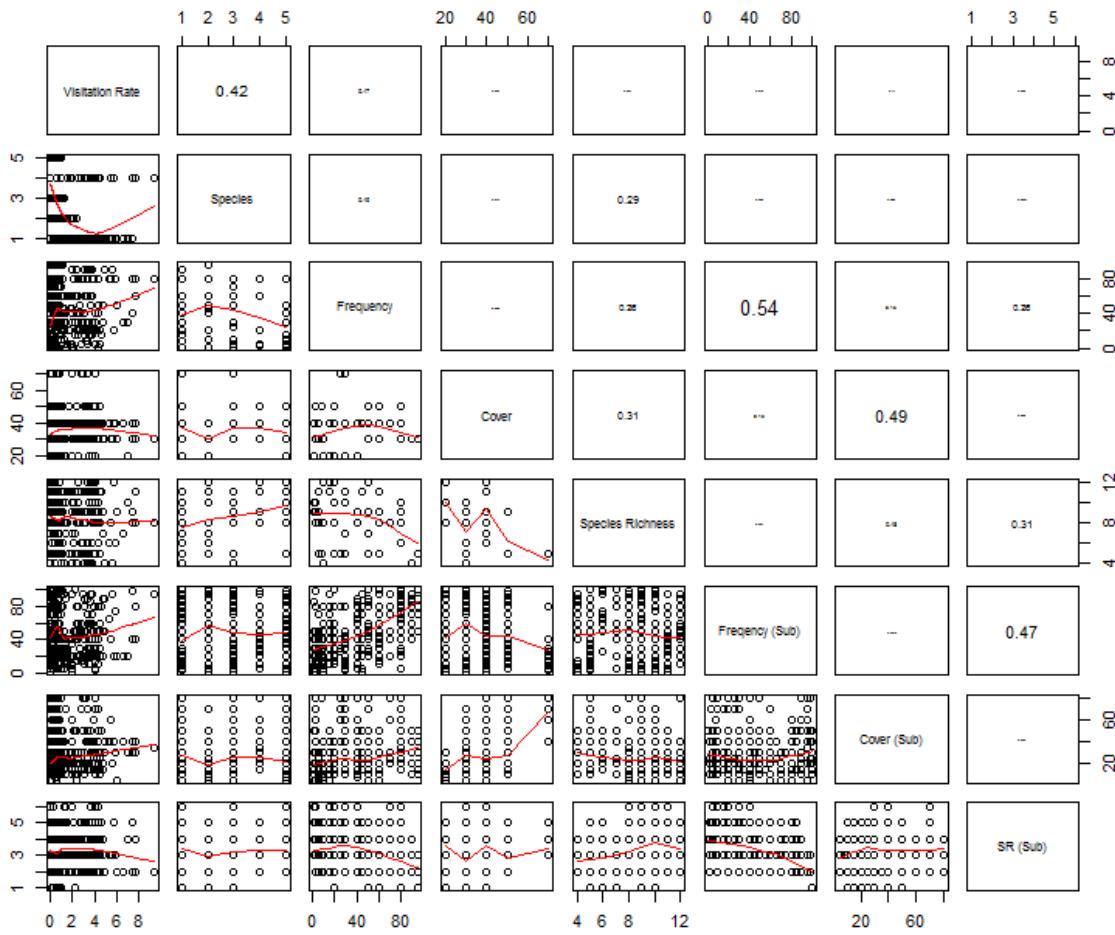


Figure S2: Pairwise correlation of per-flower visitation rate (response variable), species, frequency, floral cover and species richness on plot and subplot level. The upper panels contain Pearson correlation coefficients with its size proportional to its value. Parameters correlate on the plot and subplot level but show no strong correlation not among each other.

Table S2: Variance inflation factors (VIF) for the full set of variables. Values are calculated by the "corvif"-function from R-package AED. All values are well below three indicating no collinearity (see Zuur et al. (2007)).

Variable	GVIF
Visitation Rate	1.26
Species	1.36
Frequency	1.63
Floral Cover	1.46
Species Richness	1.45
Frequency (Subplot)	1.84
Floral Cover (Subplot)	1.37
Species Richness (Subplot)	1.48

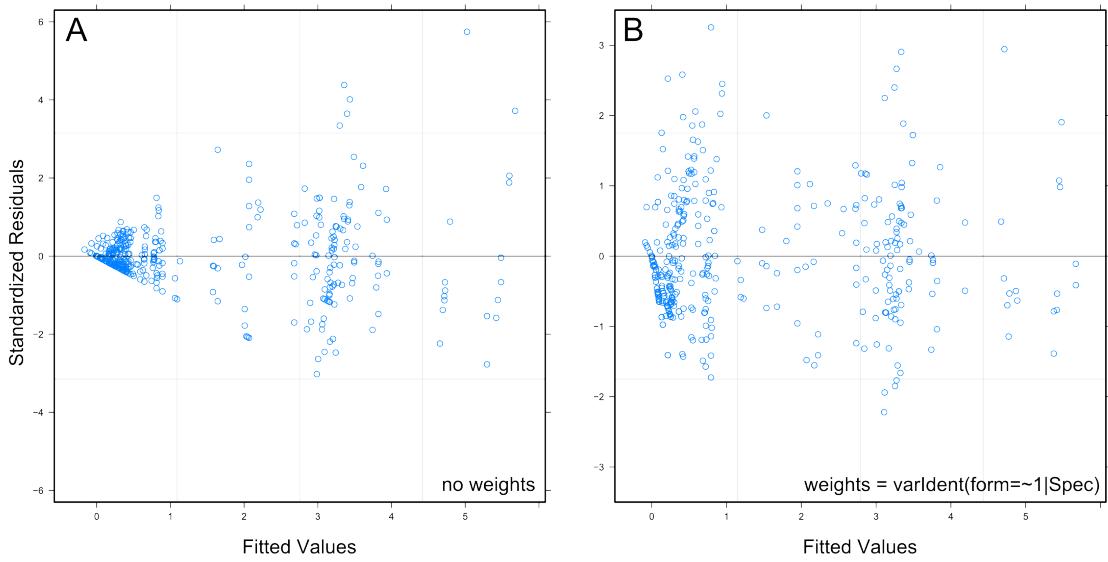


Figure S3: Standardized residuals plotted against fitted values for the final mixed effect model with and without applied weighting. A) Without weighting, the residuals show strong pattern of heteroscedasticity. B) The weighting allows different variance per species. The final model is significantly better with weighting ($L=393.32$, $df= 4$, $P < 0.0001$)

Table S3: Extended output from the linear mixed effect model. Floral cover, species richness and its interactions were removed in the model selection.

Explanatory Variables	Estimate	\pm SE	P
Intercept (Ger)	1.41	0.49	0.0048
Lat	-1.58	0.58	0.0069
Lot	-1.38	0.51	0.0075
Ono	-1.31	1.06	0.2182
TP	-1.39	0.5	0.0060
Frequency	0.11	0.05	0.0147
Frequency ²	-0.002	< 0.01	0.0699
Frequency ³	< 0.01	< 0.01	0.1174
Frequency x Lat	-0.09	0.05	0.0925
Frequency x Lot	-0.09	0.05	0.0687
Frequency x Ono	0.32	0.14	0.0183
Frequency x TP	-0.1	0.05	0.0425
Frequency ² x Lat	< 0.01	< 0.01	0.1013
Frequency ² x Lot	< 0.01	< 0.01	0.2053
Frequency ² x Ono	-0.01	< 0.01	0.0113
Frequency ² x TP	< 0.01	< 0.01	0.1479
Frequency ³ x Lat	> -0.01	< 0.01	0.0966
Frequency ³ x Lot	> -0.01	< 0.01	0.2996
Frequency ³ x Ono	< 0.01	< 0.01	0.0086
Frequency ³ x TP	> -0.01	< 0.01	0.2283

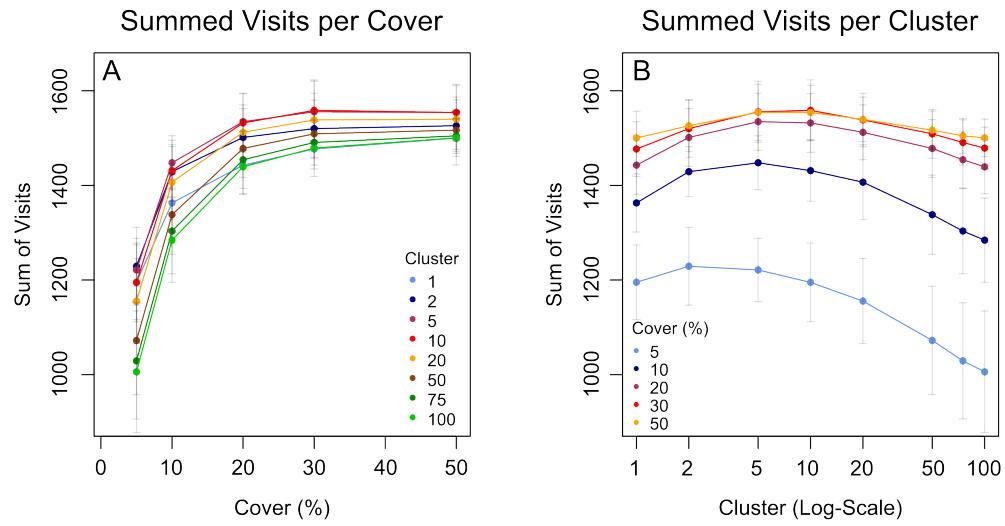


Figure S4: Frequency independent influence of floral cover and cluster size on the sum of all visits to both species together. A) Cover strongly influences the number of visits as it reduces the flight and search time and shows a saturated relationship for all cluster values. This matches the Holling's type II functional response. B) The degree of clustering influences the sum of visits in a hump-shaped function. The maximum (depending on the cover) lays between 5 and 10 flowers per cluster. A small aggregation of flowers reduces search times but keeps the next patch within visible distance. The bigger the cluster, the more difficult for the bee-agent to find the next one.

Table S4: The full set of parameters and default values used in the model.

Parameter	Description	NetLogo-Type	Type	Value	Reference
SETUP PARAMETERS:					
area	"world" in NetLogo, defined by a grid of cells called patches		integer	100x100	Kunin 1991 in Kunin and Iwasa 1996
patch-size	Size of one grid-cell in NetLogo. Can be either a flower or grass		float	0.1m ²	0.09-0.17 (Essenberg, 2012)
tick	One time-unit in NetLogo		integer	1s	Waddington 1980
flower-cover	Proportion of grid cells containing a flower	global	integer	5, 10, 20, 30, 50	Chittka et al. 1997; Kunin 1993
frequency	Proportion of flowers which are species A ($Freq_B = 100 - Freq_A$)	global	integer	0-100%	Goulson 2000, see Goulson 1999 for review
cluster-number	Average number of flowers per cluster	global	integer	1, 2, 5, 10, 20, 50, 75, 100	Dyer et al. 2008; Wertlen et al. 2008
number-bees	Initial number of pollinators in the model	global	integer	10	Né'man and Kevan 2001
BEHAVIOURAL PARAMETERS:					
search-speed	Distance a pollinator can move per time step	bees-own	integer	0.1m/sec	Kunin 1991 in Kunin and Iwasa 1996
stddev-angle	Standard deviation for the normal distribution used in the CRW	global	integer	30	0.1s (= 5 ticks)
flightsteps-until-change	Seconds of unsuccessful search before the preference changes	bees-own	integer	4	Chittka et al. 1997; Kunin 1993
length-memory	How many flowers can a bee remember to avoid double-visiting	bees-own	integer		Goulson 2000, see Goulson 1999 for review
view	Value for the radius of grid-cells a pollinator can see (cone-view of 180°)	bees-own	integer	0.7m (= 6 grid-cells)	Dyer et al. 2008; Wertlen et al. 2008
array	Array of all suitable flowers (REFERRED, non-visited) in sight of the pollinator	bees-own	Array		Né'man and Kevan 2001
reward-function	How much reward is replenished per second	flowers-own	float	0.00004 J/s	Roubik 1992 in Kunin and Iwasa 1996
handling-time	The time a pollinator needs for exploiting the floral reward	bees-own	integer	reward * 4s + 0.5s (+ 3s)	Roubik 1992 in Kunin and Iwasa 1996
reward	Reward in Joule the flower has to offer.	flowers-own	float	reward(max) = 1J	Kunin 1991 in Kunin and Iwasa 1996
pollen carry-over rate	Exploited with each visit, replenished over time	flowers-own	integer	1, 2, 4, 6, 8, 16	Benadi et al. 2012
flower-memory	Number of heterospecific visits within a successful pollination is possible	bees-own	string	4	see Goulson 1999 for review
reward-memory	A list of flower-locations	bees-own	string	4	
change-prob	A list of the last gained rewards	bees-own	float		
choice	Probability to change the preferred flower type.	bees-own	boolean		
	Increases with low reward and long search times				
	Current flower choice for the pollinator (for constancy)				

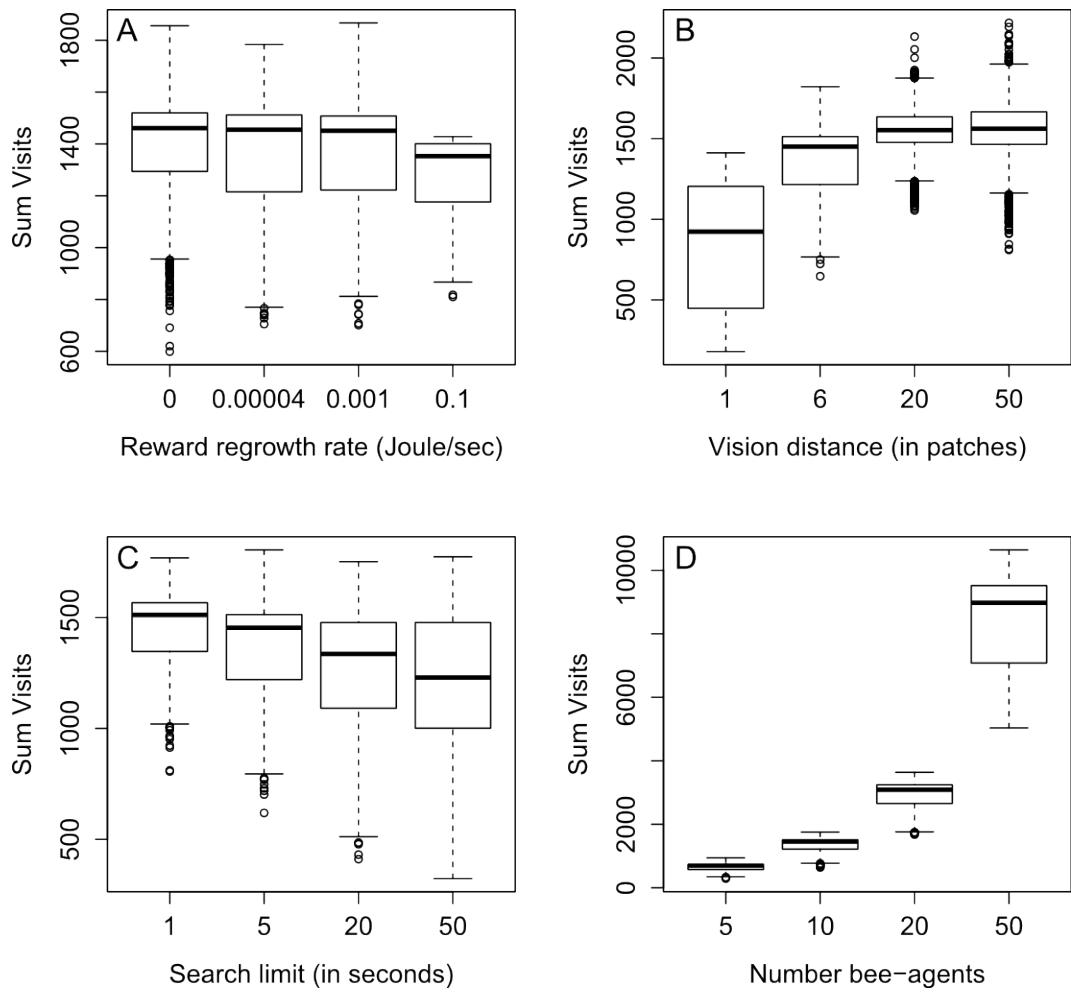


Figure S5: The influence of reward replenishment, vision, search limit and number of bee-agents on the sum of visits within a 1000 time-step simulation run. A) An unnatural high reward replenishment rate has a small negative influence on the visits. If the flowers are refilled within 10 time steps B) Bee-agents with a far field of view can detect flowers faster, move in a direct way towards them and be therefore more efficient. However, the curve is saturated at a view of 20 patches. C) An increase in search time limit decreases the sum of visits and spread the variance. Bees-agents will keep searching for rare flowers instead of switching to the common species. D) As assumed, more bees lead to more visits.

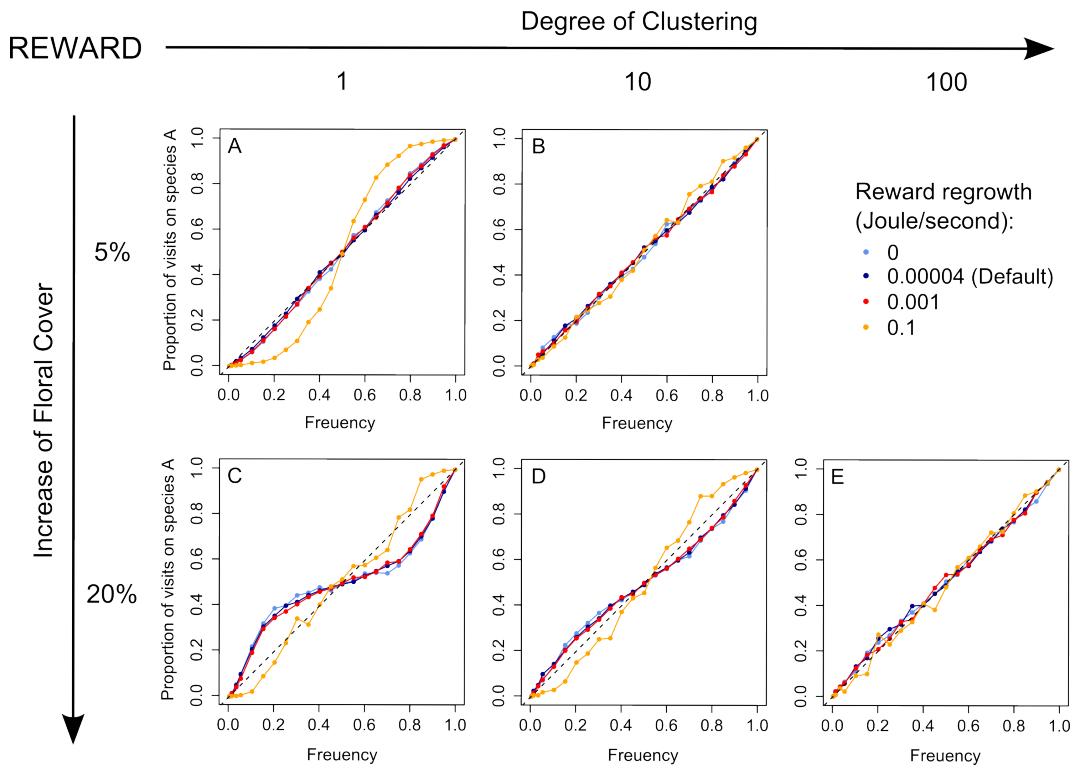


Figure S6: Outcome of the model if the reward replenishment function is changed to 0, 0.004, 0.001 and 0.1 Joules per time step. Only the unnatural high reward function (complete regrowth after 10 seconds) has an influence on the frequency dependence: the bee-agents have no more reason to change preference due to bad reward. This favors the more common species as shown in the opposite curving for no-cluster environments. A lower replenishment rate has no effect.

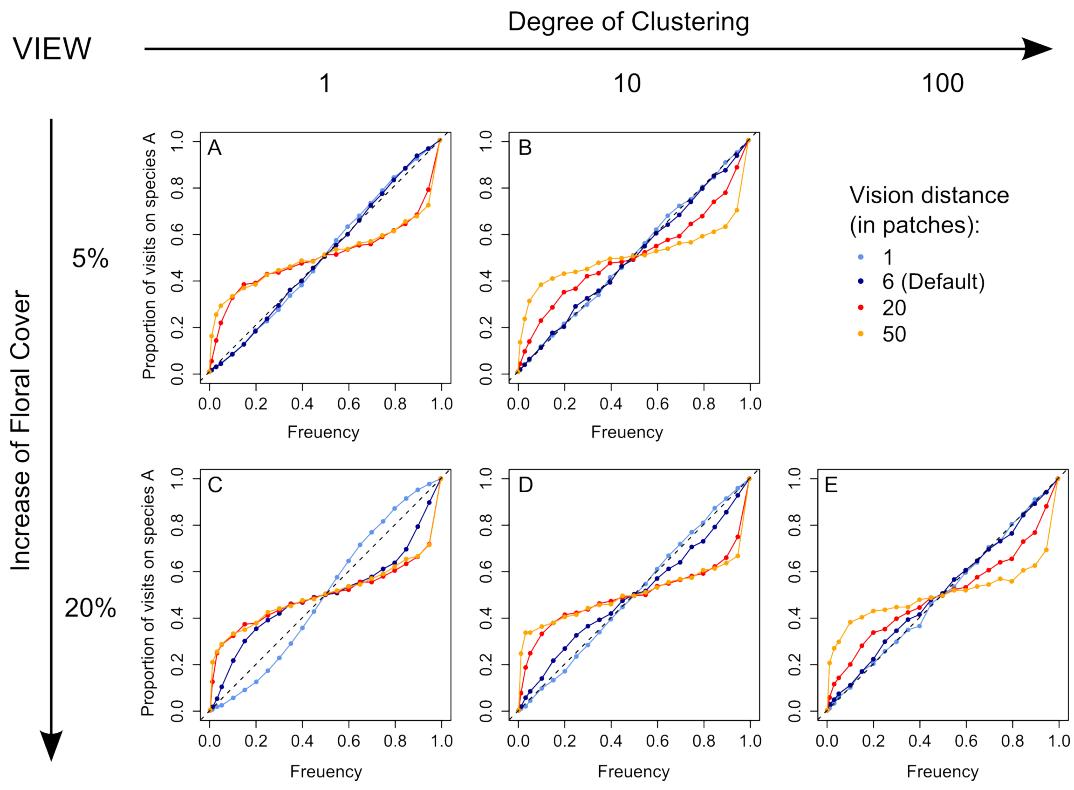


Figure S7: Effect of increased or reduced vision for the bee-agent. The vision influences the behavior of the bee-agent. If it sees far, it can move on direct way towards the next preferred flower and saves searching time. But it also flies longer distances instead of changing to the common species. A large vision therefore increases the frequency dependence and a very low vision shifts the advantage towards the more abundant species.

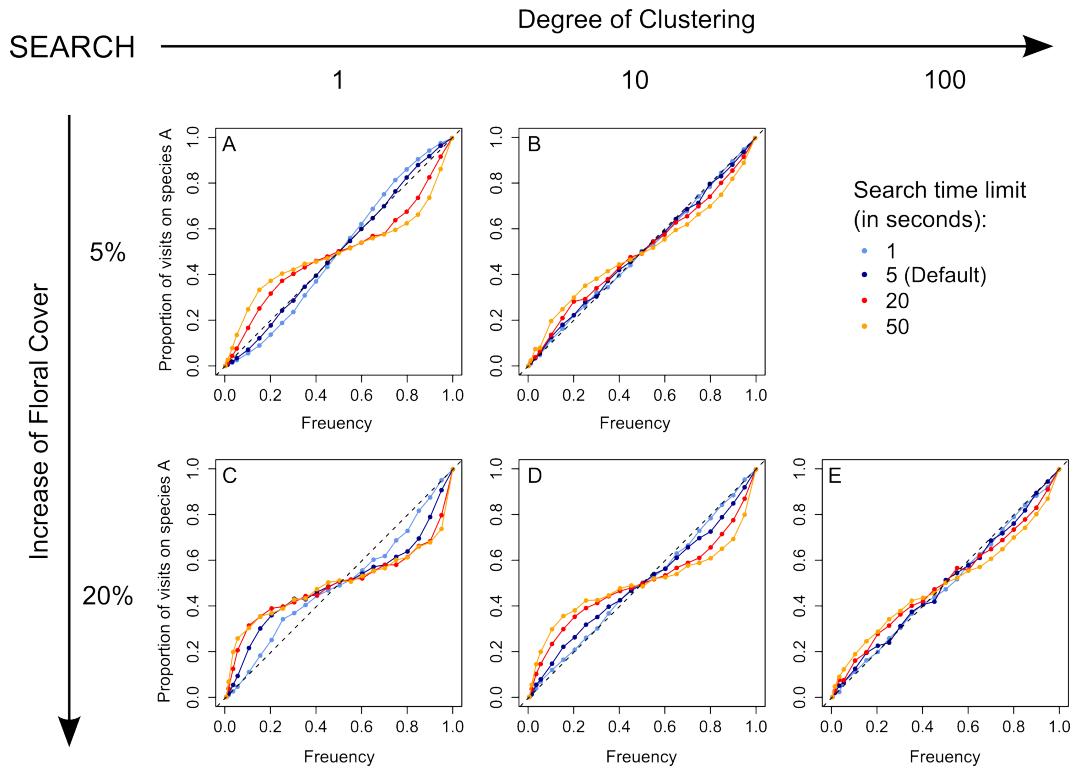


Figure S8: Sensitivity analysis for search limits of 1, 5, 20 and 50 seconds. The search limit is the number of seconds within a bee-agent searches for a unvisited and preferred flower, moving around the meadow by a correlated random walk. After the search limit is reached, the probability to change its flower preference increases with every additional second of unsuccessful search by 10%. The search limit has a similar effect on the outcome of the model as the vision because it also influences the change probability. With a higher search time, the bee-agent continues searing instead of switching to the more abundant flower, the frequency dependence is increased. A higher cluster value weakens the effect.

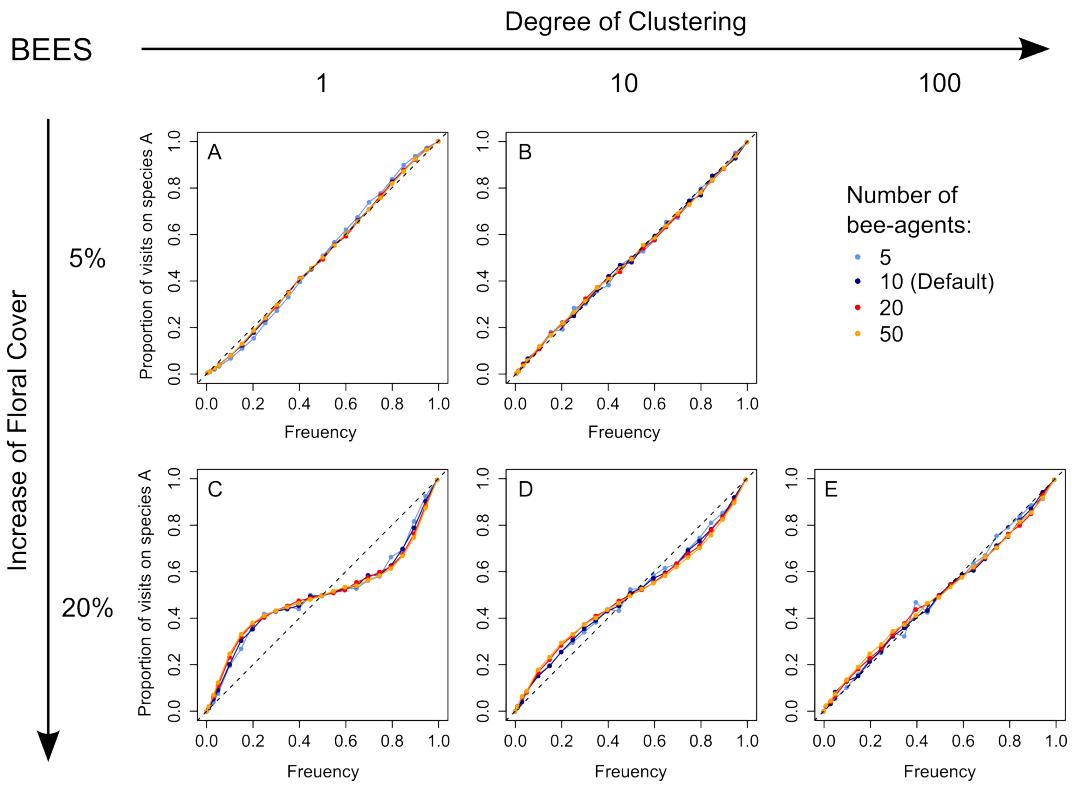


Figure S9: Results of the model for 5, 10, 20 and 50 bee-agents on the meadow. The proportion of visits does not change, only the absolute numbers. Therefore has the pollinator density no influence on the frequency dependence.